APPENDIX A

(CLEAN VERSION OF SUBSTITUTE SPECIFICATION EXCLUDING CLAIMS)

(Serial No.: 09/202,104)

IL-6 AND IL-6-RECEPTOR DERIVED PEPTIDES HAVING IL-6 ANTAGONISTIC OR AGONISTIC ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of PCT/NL97/00345, filed June 19, 1997, designating the United States of America, corresponding to PCT International Publication WO 97/48728 (published in English on December 24, 1997).

BACKGROUND

[0002] Technical Field: The invention relates to the field of cytokines.

[0003] Cytokines are substances that are produced by cells of the immune system and are involved in regulation of humoral and cellular immune reactions and inflammatory responses. Many cytokines are known, and all exert influence on various reactions in the body in a complicated fashion. To illustrate their interdependency and the intricate web of relationships that exist between cytokines, one often speaks about the "cytokine network".

[0004] Interleukine 6 (IL-6) is a cytokine which has many effects upon mammalian cells. It exerts these effects through binding to a specific cell surface receptor, that consists of a specific α-subunit of with a molecular weight of approximately 80 kD and a common β-subunit of approximately 130 kD, also named gp13O. The gpl30 β chain is also involved in signal transduction of interleukin-11. (IL-11), leukemia inhibitory factor (LIF), oncostatin M (OM), ciliairy neurotrophic factor (CNTF), and cardiotrophin-1 (CT-1) (P.B. Sehgal, Ling Wang, Ravi Rayanade et al., pp 1-14; volume 762, Annals of the New York Academy of Sciences; 1995).

[0005] IL-6 is an extremely pieiotropic cytokine, and its activities include: induction of Ig production by B cells, stimulation of B and T cell growth, differentiation of T cells and macrophages, induction of acute phase protein production by hepatocytes, multilineage hematopoesis, osteoclast formation, maturation of megakaryocytes, and platelet production. IL-6 also effects the central nervous system: IL-6 is an endogenous pyrogen and can induce ACTH production by the pituitary, finally resulting in increased qlucocorticoid levels in the circulation. IL-6 exerts its activity by triggering a transmembrane receptor that is present on all target cells. Specific steps in the IL-6 signaling cascade are the binding to the low affinity α-chain (CD126).

The complex of IL-6 and α-chain binds with the high affinity signal transducing β-chain (GP13O, CD13O).

[0006] In healthy individuals no or only very low levels of IL-6 (<10 pg/ml) are detectable in the circulation. IL-6 levels are increased in various diseases, and it is postulated that these increased levels play a causative role in the pathogenesis of these diseases. Examples of diseases where increased levels of IL-6 are found are multiple myeleoma, AIDS lymphoma, polyclonal B cell activation as observed in AIDS, rheumatoid arthritis, cardiac myxoma and Castleman's disease, mesangial proliferative glomerulonephritis, psoriasis, cancer-associated cachexia, postmenopausal osteoporosis, sepsis, multiple system organ failure, alcohol cirrhosis, and diseases of the central nervous system like Alzheimer, among others. Evidence for the causative role of IL-6 in the pathogenesis of some of the above-mentioned diseases has come from phase I/II clinical trials with IL-6 neutralizing monoclonal antibodies. Treatment with anti-IL-6 monoclonal antibodies reversed fever, acute phase proteins, night sweats, bone destruction, and cachexia. Treatment of a patient with Castleman disease with anti-IL-6 monoclonal antibodies reduced acute phase protein levels, fever, anemia, thrombocytosis, and hypergamma-globulinemia. Improvement of patients was also observed in patients with rheumatoid arthritis. Apparently, reduction of IL-6 activity in these patients resulted in improvement of the clinical signs of their disease.

[0007] This approach for treating disease by antagonizing IL-6 activity makes use of monoclonal antibodies directed to IL-6. However, monoclonal antibodies are usually not of human origin and repeated administration of non-human monoclonal antibodies generally leads to immune responses against the constant part of the antibodies, since this is foreign to the body of the patient. This immune_reaction to the monoclonal antibodies used in the treatment is, first of all, counterproductive to the therapeutic treatment itself. The monoclonal antibodies used will be rendered ineffective by the reaction with the antibodies produced by the immune system. Secondly, repeated administration of non-human monoclonal antibodies may elicit such severe immune reactions that they will be detrimental to the patient. Methods for producing less antigenic antibody fragments and methods for humanizing antibodies have been proposed, but, if feasible at all, these methods are not very economical and will their own give rise to problems

regarding to half-life and bio-availability. Consequently, using anti-IL-6 monoclonal antibodies in the treatment of IL-6 related disease is considered not to be feasible.

[0008] Inhibitors or antagonists based upon mutagenesis of IL-6 have also been proposed, such as IL-6.Q160E/T163P (Brakenhoff, J., de Hon, F., Fontaine, V., et al.; J. Biol. Chem.; 269:86-93 (1994)), and IL-6.Q159E/T162P (Ehlers, M., de Hon, D., Klaasse Bos, H, et al., J. Biol. Chem.; 270:8158-8163 (1995)). It has been shown with these mutant proteins that receptor binding of IL-6 and signal transduction of IL-6 can be separated *in vitro*. However, such mutant proteins are also foreign to the body of the patient to be treated and will also elicit an unwanted and unfavorable immune response that generally is detrimental to the treatment. Furthermore, such mutant proteins may only be partly effective, in that, although they may effectively block or inhibit specific IL-6 activities, at the same time they may exert other effects on the cytokine network with additional, still remaining, reactive sites present on these proteins. Therapeutic treatment with such reagents would then elicit other, yet unpredictable, side effects. A great disadvantage of earlier reported mutant IL-6 and IL-6 receptor antagonists is that these molecules, instead of inhibiting IL-6 *in vitro*, act as carrier and increase the half-life time and result in an increase of IL-6 activity *in vivo*. Moreover, these mutant IL-6 and IL-6 receptor antagonists have a low affinity to their target molecules and will likely act as an immunogen. In addition, antibodies raised to IL-6 stabilize IL-6 and result in an increased IL-6 production. Accumulation of circulating IL-6 as stable IL-6-anti-IL-6 complexes as a result of treatment with these antibodies to IL-6, will occur as no renal filtration can be expected. Repeated use of nonhumanized IL-6 antibodies to human patients will most likely induce antibody production to these antibodies, and result in formation of immune-complexes (Heremans, H., Dillen, C., et al. J. Immunol. 22:2395-2401).

SUMMARY OF THE INVENTION

[0009] The present invention provides a solution to the above-illustrated problems without hampering the possibility of therapeutic treatment of IL-6 related disease. The above methods to inhibit IL-6 activity by antibodies or mutants, differ greatly from the invention as described here: peptides that antagonize or agonize IL-6 at the binding site to the receptor in three ways: at the IL-6 part, at the α-receptor part, and at the gp130-receptor part. These

antagonists and agonists and combinations of these antagonists and/or agonists as multimeric peptides or as single peptides with defined pharmacokinetic characteristics gives a powerful tool to manage IL-6 bioactivity. With the solution provided by the present invention, immune responses to the treatment do not occur. Further, the occurrence of unpredictable side effects is greatly minimized.

[0010] The invention provides synthetic peptides that interact with the receptor site of IL-6 or with IL-6 receptors (α and β) present at target cells.

[0011] The invention further provides synthetic peptides that, when combined, interact with the receptor site of IL-6 as well as with IL-6 receptors (α and β) present at target cells. A mixture of these peptides is particularly valuable as the pharmacological properties of the peptides can be adjusted to obtain a maximal desired effect. Moreover, half-life time can be prolonged by inserting unnatural amino-acids into the synthetic peptides. The antagonizing or agonizing activity of the peptides is increased by producing di- or multi-meric peptides directed to one or more receptor sites. Such di- or multi-meric peptides can for instance be made by linking the peptides via one or more amino-acids such as lysine (Tam, PNAS 1988, 85: 5409-5413). The distribution of the peptides into target organs can be optimized by adjusting the hydrophilic or lipophylic nature of peptides or by binding of these peptides onto peptides that interact with specific organ markers. Finally, the peptides provided can be bound onto the solid phase of membranes or filters that are connected into an extra-corporal blood circulation circuit of the patient. A more efficient clearance of IL-6 and/or soluble IL-6 receptors can in that way be achieved.

[0012] Such synthetic peptides can be derived from (A) IL-6, or derived from (B) the receptor α-chain of IL-6 (IL-6Rα, CD126), or from (C) the receptor β-chain of IL-6 (IL-6Rβ, GP130, CD130) and exhibit antagonistic and agonistic activity against the various components and steps of the IL-6 signaling cascade. The peptides were found by testing sets of overlapping amino acid sequences from the published human ILE (Hirano, T., Yasukawa, K., Harada, H., et al.; Nature 324, 73-76 (1986); Yasukawa, R., Hirano, T., Watanabe Y., et al.; EMBO J. 6:2939-2945 (1987), IL-6Ra (Yamasaki, K., Taga, T., Hirata, Y., et al.; Science 241:825-828 (1988)) and IL-6Rβ (Hibi, M., Murakami, M., Saito, M.; Cell 63:1149-1157 (1990)). These

overlapping peptides, each twelve amino acids long, were tested in an assay for antagonistic or agonistic IL-6 activity.

[0013] The peptides provided by the invention all exhibit antagonistic or agonistic IL-6 activity against the IL-6 signaling cascade as measured in an IL-6 assay. The peptides of the present invention are too small to generate immune responses. Further, they are too short to contain additional reactive sites, so that the antagonistic and, in addition, the agonistic peptides can advantageously be used to treat patients to counteract and adjust elevated IL-6 levels. The amino acids in all antagonistic or agonistic peptides described below are identified by the one letter code, in which the N-terminal (head) amino acid is listed first (on the left) and the C-terminal (tail) amino acid is listed last (on the right).

[0014] A. The antagonistic peptides derived from IL-6 preferably comprise at least 5 consecutive amino acids selected from the following 3 regions that were identified as RYILDGISALRK (SEQ ID NO:1), STKVLIQFLQKKAKNL (SEQ ID NO:2), and ILRSFKEFLQSSLRALRQM (SEQ ID NO:3).

[0015] B. The antagonistic peptides derived from the receptor α-chain of IL-6 preferably comprise at least 5 consecutive amino acids selected from the following 3 regions that were identified as QLSCFRKSPLSNVVC (SEQ ID NO:4), PRSTPSLTTKAVLLVRKFQNS (SEQ ID NO:5), and MCVASSVGSKFSKTQTFQGC (SEQ ID NO:6). The agonistic peptides derived from the receptor α-chain of IL-6 preferably comprise at least 5 consecutive amino acids selected from the following region that was identified as EWGPRSTPSLTTKAVLLVRKFQNSPAED (SEQ ID NO:11).

[0016] C. The antagonistic peptides derived from the receptor β-chain of IL-6 preferably comprise at least 5 consecutive amino acids selected from the following 4 regions that were identified as PEKPKNLSCIVNEGKKMRCEWDGGR (SEQ ID NO:7), NFTLKSEWATHKFADCKAKRDTPTS (SEQ ID NO:8), WVEAENALGKVTSDH (SEQ ID NO:9), and PVYKVKPNPPHNLSVIN (SEQ ID NO:10).

[0017] Relatively short peptides (as short as a string of 5 amino acids) that are selected from any of the above peptides, or peptides of no more than 30 amino acids long which show antagonistic or agonistic activity as measured in an IL-6 assay and have at least one string of at least 5 amino acids in common with the peptides from groups A, B or C, are also peptides

of the present invention. The peptides according to the invention can vary in length. Also, the peptides comprising a string of at least 5 amino acids which are in common with the peptides from groups A, B, and C can be modified by replacing one or a few amino acids in said string by other amino acids. Such amino acids can be selected from any of the naturally occurring amino acids, but also amino acids that normally do not occur in nature can be used as replacement amino acids. The choice of the replacing amino acid can, for example, be guided by comparing IL-6 or IL-6 receptor sequences from other species than humans or by selecting amino acids that lead not to extreme functional or conformational changes of the selected peptide, but also other selection methods can be used. More in particular, the present invention relates in a first aspect to a peptide containing at least 5 amino acids and at most 30 amino acids that exhibits antagonistic activity directed against IL-6 and/or against the α-chain of the IL-6 receptor and/or against the β-chain of the IL-6 receptor.

[0018] Also, the present invention relates in another aspect to a peptide containing at least 5 amino acids and at most 30 amino acids that can exhibit antagonistic or agonistic IL-6 activity, depending on the concentration in which it is used. An example of such peptides are peptides selected with as basis with the amino acid sequence EWGPRSTPSLTTKAVLLVRKFQNSPAED (SEQ ID NO:11) as found in the α-chain of the IL-6 receptor. Surprisingly, peptides selected on the basis of the aforementioned sequence expressed antagonistic IL-6 activity at high concentrations whereas at low concentrations a marked agonistic activity was found. Agonistic activity was observed in the *in vitro* bioassay in a concentration range from 7.5 to 120 μg/ml peptide. At a concentration of ≥120 μg/ml these peptides had an antagonistic effect upon the biological activity of IL-6 in the bioassay. The agonistic peptides can be used *in vitro* in concentrations that are relatively equivalent but not necessarily the same as when used *in vitro*.

[0019] Furthermore, the invention provides combinations of peptides, either provided as a simple mixture of several, possibly modified, peptides selected from groups A, B or C, or, provided as, possibly modified, peptides selected from groups A, B, or C that are linked, with direct chemical bonds or using spacer molecules, head to tail, or head to head, or tail to tail, or via side chains of the amino acids present in the selected peptides.

[0020] Examples of such combinations of peptides are, for example, using the peptides SLTTKAV (SEQ ID NO:15) and ILRSFKEFLQSS (SEQ ID NO:16), or WVEAENALGKVTSDH (SEQ ID NO:17) and RYILD (SEQ ID NO:18), or KAVLLVRK (SEQ ID NO:19), but many other combinations of two or more peptides can be selected from the peptides listed in groups A, B or C. Such combinations of peptides, be it simple mixtures or bound peptides, can advantageously be used to counteract the events occurring in the IL-6 signaling cascade, such as disrupting the binding of IL-6 to the α-chain by simultaneously competing at both the IL-6 and the α-chain binding site, or simultaneously competing at the binding sites of the IL-6/α-chain complex and the β-chain.

[0021] The peptides of the invention can suitably be used in a medicinal or pharmaceutical preparation for therapeutic or prophylactic purposes. Further, they can be used in protocols to remove circulating IL-6 from the blood of diseased patients via dialysis methods in which the peptides are bound to a solid phase. Passing blood or blood filtrates along the thus bound peptides will result in clearance of IL-6 that will bind to the peptide at the solid phase. Also the peptides according to the invention may be added to blood or blood filtrates and (ir) - reversibly bind to IL-6 or IL-6 receptor molecules and thus render these inactive before they re-enter the body. Also, the peptides can be used in diagnostic tests, i.e., in direct binding or competition based enzyme-linked immunosorbent assays to measure IL-6 levels.

DETAILED DESCRIPTION OF THE INVENTION

[0022] IL-6 agonistic peptides can completely or partially replace IL-6 that is added to cell-cultures, for example, IL-6 is used to grow or culture IL-6 dependant cells, like B-cell hybridomas to which IL-6, as growth factor, is often added. In addition, cell-cultures in general will benefit from the addition of agonistic IL-6 peptides. The IL-6 agonistic peptides administered to humans or animals can be used to enhance the immune response of an host exposed to a specific immunogenic substance. The IL-6 agonistic peptides can be administered to humans or animals to increase the responsiveness of the immune system of the host. A specific use is in pharmaceutical preparations for topical or intramammary application. When these agonistic peptides are combined with IL-6 antagonists, as described, excess of IL-6 can be inhibited without loss of basal IL-6 signal transduction.

[0023] Antibodies specifically directed against the peptides, and their corresponding anti-idiotypic antibodies, are part of the invention. Such antibodies can, for example, be administered to patients treated earlier with the peptides, to counteract the effect of the peptides on the patient. Such antibodies can be used in the above-described dialysis protocols and diagnostic tests.

[0024] Synthesis of the peptides may be accomplished according to the available methods in the art. The synthesis of the exemplified peptides was done according to Valerio et al. (Int. J. Peptide Res., 42:1-9 (1993) and/or Valerio et al. (Int. J. Peptide Res., 44:148-165 (1994)). Methods for large scale production of synthetic peptides and the purification thereof are well known in the art. The invention is illustrated in the following experimental part.

Brief Description of Figures:

[0025] Fig. 1 Amino acid sequences and sources of selected peptides (SEQ ID NOs:12-14, FIGs. 1A-1C, respectively);

[0026] Fig. 2 Screening of synthesized peptides, representing IL-6, in the B9 bio-assay;

[0027] Fig. 3 Screening of synthesized peptides, representing IL-6Ra, in the B9 bio-assay;

[0028] Fig. 4 Screening of synthesized peptides, representing gp130, in the B9 bio-assay; and

[0029] Fig. 5 Screening synthetic peptides showing a region with agonistic activity (dilution 1:50).

Experimental part

1. Peptide synthesis.

[0030] The peptides of the examples which were intended for identifying active centers in the IL-6 and IL-6 receptor molecules were synthesized using a method according to Valerio et al. (Int. J. Peptide Res., 42:1-9 (1993) and/or Valerio et al. (Int. J. Peptide Res., 44:148-165 (1994)). Multimeric peptides (four branched) were synthesized by the solid-phase method, using a dispersed system with branching oligolysines as a scaffolding for incorporation of the synthesized antagonistic peptides (Tam, J.P.; Proc. Natl. Acad. Sci. USA, 85:5409-5413 (1988).

2. Proliferation assay to determine antagonistic IL-6 activity.

[0031] A set of overlapping peptides, each twelve amino acids long (each consecutive peptide shifts one amino acid, so consecutive peptides have 11 amino acids in common), derived from human IL-6 sequence (Hirano, T., Yasukawa, K., Harada, H., et al.; Nature 324, 73-76 (1986); Yasukawa, R., Hirano, T., Watanabe Y., et al., EMBO J. 6:2939-2945 (1987), were incubated with cells (B9) at 37°C. After one hour, recombinant human IL-6 (CLB, Amsterdam, The Netherlands) was added at 3 different concentrations (2.5 U/ml, 5 U/ml and 10 U/ml).

[0032] A set of overlapping peptides, each twelve amino acids long (each consecutive peptide shifts one amino acid, so consecutive peptides have 11 amino acids in common), derived from human IL6Ra (Yamasaki, K., Taga, T., Hirata, Y., et al.; Science 241:825-828 (1988)) or qpl30 (Hibi, M., Murakami, M., Saito, N.; Cell 63:1149-1157 (1990)), were incubated with 3 different concentrations IL-6 (2.5 U/ml, 5 U/ml, 10 U/ml) diluted in DMEM supplemented with HT for one hour at 37°C. Then the residual IL-6 activity was determined in a biological assay by measuring the IL-6 dependant proliferative growth of B9 mouse hybridoma cells (Helle, M., Boeije, L., Aarden, L.A.; Eur. J. Immunol. 18:1535-1540 (1988)). Briefly, B9 mouse hybridoma cells were collected during their logarithmic growth phase in IL-6 free media and suspended at a concentration of $1*10^5$ cells/ml in DMEM+HT medium containing 5% FCS. Fifty μl of each IL-6 dilution was combined with each of the synthesized peptides representing IL-6 sequences and incubated for 1 hour at 37°C. This mixture was added in duplicate to 50 μl of the B9 cell suspension in flat-bottomed 96-well tissue culture plates (Greiner) and incubated at 37°C and 5% CO_2 for 72 h. IL-6 activity was assessed by using 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT, Sigma). After addition of 25 μl of MTT (5mg/ml dissolved in PBS) to each well and further incubation at 37°C for 4 h, 100 μl of lysis buffer (20% w/v SDS in 50% dimethyl formamide) was added. Thereafter, incubation was continued over night at 37°C and the next morning absorbance was read at 578 nm.

[0033] To determine the agonistic or antagonistic activity to IL-6 of the peptides synthesized from the sequences of the IL-6 receptor α or β, various concentrations of each of these peptides was combined with 50μl of the B9 cell suspension ($1*10^5$ cells/ml in DMEM+HT medium containing 5% FCS). This suspension was incubated for 1 hour at °37C, and combined

with each of the dilutions of IL-6 into flat-bottomed 96-well tissue culture plates (Greiner). Plates were incubated at 37°C for 72 h. IL-6 activity was assessed as described above.

[0034] Samples without synthetic peptides or with a sham peptide but with IL-6 were used as positive control, whereas samples that contained neither IL-6 nor synthetic peptides were used as negative control. Inhibition or enhancement of IL-6 activity was determined by calculating the ratio absorbance of test sample and absorbance positive control both corrected for negative control absorbance.

3. Toxicity testing of peptides.

[0035] Three separate tests were performed to determine whether the synthetic peptides exert toxic effects in vitro upon erythrocytes (A), or polymorphonuclear cells (B), or hepatocytes (C).

[0036] A. Sheep red blood cells (SRBC) were washed five times in PBS. A 1% (v/v) suspension of erythrocytes was prepared in veronal-buffered saline that contained gelatin (GVS: 0.032% gelatin in 3.9 mM barbitone sodium, 1 mM $MgSO_4$, 0.38 mM $CadI_2$, and 145.6 mM NaCl). Twofold dilutions of the synthetized peptides (50 μl) were made in U-shaped microtiter plates (Greiner Labortechnik) and 50 μl of the SRBC suspension were added to each well. Plates were sealed, mixed and incubated for 2 hours at 37°C. Thereafter, plates were examined for hemolysis. None of the synthetized peptides showed hemolysis.

[0037] B. Porcine polymorphonuclear cells (PMN) were isolated from pig blood (Cruijsen, T.L.M., Van Leengoed, L.A.M.G. et al.; Infect. Immun. 60:4867-4871 (1992)). Twofold dilutions of the synthetic peptides (50 μl) were made in flat-bottomed microtiter plates (Greiner Labortechnik) and 50 μl of the PMN suspension ($2*10^6$ cells/ml) were added to each well. Plates were sealed, gently mixed and incubated for 6 hours at 37°C and 5% CO_2. Thereafter, plates were examined for cytotoxicity by nigrosine dye exclusion. None of the synthetic peptides were toxic for PMN.

[0038] C. Porcine hepatocytes were isolated from liver of pigs based on Seglens' method (Seglen, P.O.; Methods Cell Biol 13:29-83 (1976)) and adapted according to Monshouwer M., et al. (Toxicol. Applied Pharmacol. in press). Hepatocytes were suspended in Williams' medium E to a concentration of 10^6 cells/ml. From this suspension 1.5 ml was put

into each well of 12-well tissue culture plates (Costar) and incubated for 12 h at 37°C. Adherent hepatocytes were examined for their viability and non-adherent hepatocytes were discarded. Each synthetic peptide was mixed with Williams' medium E (at dilutions of 1:50 and 1:100) and added to wells with adherent hepatocytes. After another 24 h incubation at 37°C viability was assessed by using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma). After addition of 1.5 of MTT (1-mg/ml dissolved in Williams' medium E) to each well and further incubation at 37°C for 30 min, 1 ml of lysis buffer (0.8 M HCL in isopropanol) was added. Thereafter, plates were mixed for 10 min and absorbance was read at 560 nm. None of the synthetized peptides proved to affect the viability of the hepatocytes.

4. Effect of IL-6 antagonistic peptides upon IL-6 induced acute phase reaction and down regulation of hepatic biotransformation activities.

[0039] Porcine hepatocytes were isolated from liver of pigs based on Seglens' method (Seglen, P.O.; Methods Cell Biol 13:29-83 (1976)) and adapted according to Monshouwer M., et al. (Toxicol. Applied Pharmacol. in press). Hepatocytes were suspended in Williams' medium E to a concentration of 10^6 cells/ml. From this suspension 1 ml was put into each well of 12-well tissue culture plates (Costar) and incubated for 12 h at 37°C. Adherent hepatocytes were examined for their viability and nonadherent hepatocytes were discarded. Each synthetic peptide was mixed (at dilutions of 1:50 and 1:100) with Williams' medium E containing IL-6 (1000 U/ml) and added to wells with adherent hepatocytes. Also a negative (containing no IL-6 and without synthetized peptides in the medium) and positive control (containing 1000 U/ml IL-6 in the medium) were prepared and tested. After an incubation period of 24 hours, the medium was removed and for each well CYP450 dependent enzyme activity of intact monolayers of hepatocytes was determined.

[0040] CYP450 enzyme assay. CYP450 dependent enzyme activity, using testosterone (250/μM) as substrate, was determined as previously described by Van 't Klooster et al. (Bioch. Pharmacol. 46;1781-1790 (1993)). Briefly, testosterone was mixed with Williams' medium E without fetal calf serum and added to the wells with hepatocytes. After 30 min incubation at 37°C and 5% CO_2, hydroxylated testosterone metabolites in the medium were quantified by HPLC.

[0041] HPLC analysis. Aliquots of 1 ml of medium was mixed with 100 µl of a solution of 11β-testosterone (12,5 µg/ml) in methanol as internal standard and extracted with 5 ml dichlormethane. The organic phase was transferred to clean tubes and evaporated to dryness at room temperature under a stream of nitrogen. The residues were dissolved in 130 µl 50% methanol and 20 µl of these solutions were injected for HPLC analysis. The stationary phase consisted of a C18 glass column (20 cm, 3µm particle size, Chrompack, Middelburg, the Netherlands). The mobile phase consisted of buffer A (12% methanol, 75% milli Q water) and buffer B (64% methanol, 6% acetonitril, 30% milli Q water). With these buffers, an elution gradient was generated; 10-58% B from 0-45 minutes; 58-59% from 45-50 minutes; 59-10% from 50-53 minutes, with a flow rate of 0,8 ml/min. Metabolites were detected spectrophotometrically at 254 nm. Inhibition of IL-6 dependant downregulation of cytochrome P450 was determined by comparing the relative concentration of hydroxylated testosteron metabolites in medium from adherent hepatocytes incubated with synthetized peptides and IL-6, and the relative concentration of hydroxylated testosteron metabolites in medium from positive and negative control hepatocyte monolayers.

5. Results.

[0042] Peptides derived from hIL-6, hgp130 (the α-chain of the IL-6 receptor) and hIL6Ra (the α-chain of the IL-6 receptor) were analyzed for antagonistic IL-6 activity.

[0043] For hIL-6 peptides, 3 regions were identified which inhibited IL-6 activity in an IL-6 assay (FIG. 2). Peptide 31, 119-123 and 167-174 represent the identified regions (RYILDGISALRK (SEQ ID NO:1), resp. STKVLIQFLQKKAKNL (SEQ ID NO:2), resp. ILRSFKEFLQSSLRALRQM (SEQ ID NO:3)).

[0044] For hILGRa, also 3 regions were identified which inhibited IL-6 activity in an IL-6 assay (FIG. 3). Peptide 6-9, 24-33 and 80-89 represent the identified regions (QLSCFRKSPLSNVVC (SEQ ID NO:4), resp. PRSTPSLTTKAVLLVRKFQNS (SEQ ID NO:5), resp. MCVASSVGSKFSKTQTFQGC (SEQ ID NO:6)).

[0045] For hgpl30 peptides, 4 regions were identified which inhibited IL-6 activity in an IL-6 assay (FIG. 4). Peptide 2-15, 33-46, 73-76 and 92-97 represent the identified regions (PEKPKNLSCIVNEGKKMRCEWDGGR (SEQ ID NO:7), resp.

NFTLKSEWATHKFADCKAKRDTPTS (SEQ ID NO:8), resp. WVEAENALGKVTSDH (SEQ ID NO:19), resp. PVYKVKPNPPHNLSVIN (SEQ ID NO:10)).

[0046] The identified peptides with anti-IL-6 activity were not lytic for erythrocytes and not toxic for polymorpho-nuclear cells and not toxic for primary hepatocyte culture cells.

[0047] Peptides derived from hIL6Ra (the α-chain of the IL-6 receptor) were analysed for agonistic IL-6 activity and 1 region was identified which stimulated proliferation of B9 cells without IL-6 added to the medium and enhanced IL-6 activity in the B9 bio-assay (FIG. 5) . Peptide 21-37 represent the region EWGPRSTPSLTTKAVLLVRKFQNSPAED (SEQ ID NO:11) of the IL-6Ra sequence

[0048] Agonistic activity was observed in a concentration range from 7.5 to 120 μg/ml peptide. These peptides induced proliferative growth of the IL-6 dependant cell line B9, and when combined with IL-6 enhanced proliferation of the B9 cell line was examined1 and thus the biological activity of IL-6 was enhanced. At a concentration of ≥120 μg/ml these agonistic peptides had an antagonistic effect upon the biological activity of IL-6.

[0049] The identified peptides with agonistic IL-6 activity were not lytic for erythrocytes and not toxic for polymorphonuclear cells and not toxic for primary hepatocyte culture cells.

[0050] Synthesized peptides from the regions PVYKVKPNPPHNLSVIN_(SEQ ID NO:10), WVEAENALGKVTSDH (SEQ ID NO:9), and MCVASSVGSKFSKTQTFQGC (SEQ ID NO:6) inhibit IL-6 regulated down regulation of cytochrome P-450 of hepatocytes.